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### High CD8 Cell Doses Correlate with Reduced Relapse Risk and Improved Survival after Allogeneic Peripheral Blood Stem-Cell Transplantation with Reduced-Intensity Conditioning

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**Background:** Disease relapse remains the most common cause of death after allogeneic hematopoietic stem-cell transplantation (SCT). Specifically, prevention of relapse in transplants performed after reduced intensity conditioning (RIC) relies on a donor derived graft-versus-tumor (GVT) effect, which is primarily mediated by T-cells. We hypothesized that a high graft T-cell dose enhances GVT and improves outcomes. We therefore analyzed the impact of graft composition on transplant outcomes in patients undergoing allogeneic peripheral blood SCT with a uniform RIC regimen.
**Methods:** We studied 183 consecutive patients who underwent a first allogeneic SCT with peripheral blood stem-cells and fludarabine (120mg/m$^2$) + busulfan (6.4mg/kg) conditioning. Patients were allografted at the University of Pennsylvania between August 2006 and March 2013. Doses of CD3, CD8, CD4 and CD34 cells in the graft were determined by standard methods. Univariate analyses of outcomes were performed using cumulative incidence and Cox regression analyses. Multivariable models were constructed using the backward elimination method, including variables with $p<0.1$.
**Results:** The median follow-up was 20.2 mo. (range 0.4 – 78). Patients had a median age of 62 (range 21-76) and diseases included AML (71), MDS (45), NHL (38), CLL (9), ALL (5) and others (15). Mean cell doses were CD3: 2.2x10$^8$/kg (range 0.17-5.5), CD8: 0.38x10$^8$/kg (range 0.03 – 1.49), CD4: 0.96x10$^8$/kg (range 0.1 – 3.05) and CD34: 5.8x10$^6$/kg (range 1.2-21.4).

A univariate analysis of overall survival showed a trend for improved survival in patients with high CD8 cell doses (HR=0.51, 95%CI [0.24, 1.09], p=0.08). The total nucleated cell dose significantly correlated with overall survival (HR=0.95, [0.90, 0.99], p=0.04). In a multivariate model CD8 cell doses significantly correlated with improved survival (HR=0.41, [0.19, 0.91], p=0.03).

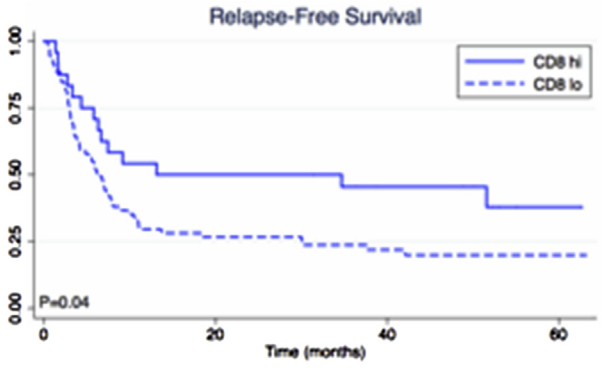


**Figure 1.** The impact of CD8 cell dose on RFS

Similar analyses of the relapse risk and relapse free survival revealed a significant protective effect of high CD8 cell doses (HR=0.36, [0.15, 0.90], p=0.03 and HR=0.46, [0.23, 0.93], p=0.03, respectively). Figure 1 illustrates the impact of high (>0.7) and low CD8 cell doses on relapse-free survival.

Acute graft-versus-host disease (GVHD) did not correlate with T-cell doses, but the risk for moderate-severe chronic GVHD was higher in patients who received high CD8 cell doses without reaching statistical significance (HR=3.35, [0.93, 12.05], p=0.06).
**Conclusion:** CD8 dose predicts clinical outcome in patients undergoing peripheral blood RIC SCT. A higher CD8 cell dose is a primary predictor of a lower relapse rate and improved relapse-free and overall survival, while there is a possible increase in chronic GVHD. Targeting high CD8 cell doses should be considered in prospective trials of reduced intensity conditioned SCT.

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### Subsequent Malignant Neoplasms after Allogeneic Hematopoietic Stem Cell Transplantation Using Reduced-Intensity Conditioning and Outpatient Conduction

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Patients given allogeneic hematopoietic transplants (HSCT) may develop subsequent malignant neoplasms (SMN). Several variables have been identified but there are no data about the incidence of this complication in individuals given HSCT using reduced-intensity conditioning (RIC) methods. The objective of this study is to define the incidence of SMN in patients given HSCT using a RIC preparative regimen conducted on an outpatient basis. Patients given HSCT in two institutions between October 1998 and 2012 were analyzed. Overall survival was analyzed with the Kaplan-Meier procedure. Patients alive at the closure of the study or those lost to follow up were censored. To appraise the SMN appearance, those patients dead were also regarded as censored at that moment, as well as those lost to follow up and those alive at the closing of the study. 95% confidence intervals for the survival or failure estimate were calculated with the Greenwood's method. All the survival analyses were processed with the StataCorp 2005. Stata Statistical Software: Release 9. College Station, TX: StataCorp LP. A total of 416 allografted patients with a Karnofsky performance index of 100% were included in the study. All patients received PBSC allografts. Engraftment occurred in 350 patients (84%). The conditioning regimen was delivered as an outpatient procedure in all individuals. No patient was given radiotherapy nor antithymocyte globulin during the conditioning. Three hundred and sixty five patients (88%) were never admitted to the hospital, whereas 12% were admitted because of grade III-IV aGVHD, fever or mucositis. Median survival time was 15.7 months. Survival at 6 months (95% CI): 66.4% (61.5- 70.8%), at 12 months: 53.3% (48.1 -58.1%), at 60 months: 30.6%

(30.5-41.5%). Eight patients with a SMN were identified in the group of 416 allografted patients, the cumulative probability of SMN being 6.8 at 10 years. Since the number of expected cases in the general population is 0.62, the ratio of observed to expected cases is 3.2 ($p < 0.001$). This figure means that the risk of developing a malignant neoplasm in allografted individuals using our method is 3.2 times higher than that in the general population. There were three non-Hodgkin's lymphomas (NHL), two M2 acute myelogenous leukemias (AML), one hairy cell leukemia, one tongue epidermoid carcinoma and one breast carcinoma. In conclusion, we have found a low incidence of SMN in this group of Mexican patients allografted with the Mexican reduced-intensity conditioning method. Possible explanations for this difference are discussed, focusing on the RIC preparative regimen.

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### Reduced Intensity Fludarabine and Intravenous Busulfan (FB2) for Allogeneic Peripheral Blood Stem Cell Transplantation

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**Objective:** To demonstrate the efficacy and safety of a reduced intensity conditioning (RIC) regimen comprising Fludarabine and intravenous Busulfan (FB2) in patients undergoing allogeneic peripheral blood stem cell transplantation (PBSCT) for hematological disorders.

**Patients and Methods:** We conducted a retrospective analysis of 42 patients who underwent RIC prior to PBSCT at our institution between the years 2009 and 2012 inclusive. FB2 consisted of Fludarabine 30 mg/m$^2$/day infused over 30 minutes for 5 days on days -6 through -2 and Busulfan 3.2 mg/kg/day on days -3 and -2 (infusion rate 80 mg/kg/hour). All patients received Thymoglobulin at a total dose of 4.5 mg/kg or 6 mg/kg administered in divided doses on days -2, -1 and 0. Post-transplantation graft versus host disease (GVHD) prophylaxis consisted of tacrolimus and mycophenolate mofetil. Diagnoses included Acute Myeloid Leukemia (n=3), Acute Lymphoblastic Leukemia (n=2), Myelodysplastic Syndrome (n=10), Severe Aplastic Anemia (n=5), Chronic Lymphocytic Lymphoma (n=8), Non-Hodgkin's Lymphoma (n=8), Hodgkin Lymphoma (n=4) and Myeloproliferative Disorder (n=2). The median age of the recipients was 56 years with 18 patients (44%) aged > 60 years. Only 9 patients (21%) were in complete remission (CR) at the time of HSCT and 19 (45%) were considered to have high-risk disease by CIBMTR criteria. The co-morbidity index was 3 or more in 19 recipients (45%).

**Results:** At a median follow up of 15 months, overall survival (OS) for the entire cohort was 62%. OS for patients undergoing PBSCT in CR versus not in CR was 89% and 55% respectively. OS was similar in recipients undergoing PBSCT from matched unrelated (n=29) or matched related (n=13) donors. Median engraftment time for neutrophils (ANC >500) and platelets (>20K) was 18 and 17 days, respectively. Acute GVHD grade 2 developed in 15 (36%) of recipients, grade 3 in 2 (5%) and grade 4 in 2 (5%). The cumulative incidence of relapse was 37% (n=13). There were no graft failures and treatment related mortality (TRM) was 2% (n=1). At one year following PBSCT, 10 patients (24%) had extensive chronic GVHD and 19 patients (45%) required continuation of immunosuppressants. All patients with SAA (n=5) are alive, engrafted and did not develop grade 3 or 4 acute GVHD.

**Conclusion:** Our preliminary data demonstrates low toxicity and favorable outcome in older patients with elevated co-morbidity score and high-risk disease using the FB2 regimen. The efficacy, tolerability and excellent outcome of FB2 warrants further study in recipients with SAA.

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### Utility of CMV PCR in the Evaluation of Allograft Recipients Presenting with Diarrhea

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**Introduction:** Graft Versus Host Disease (GVHD) and immunosuppression in allogeneic hematopoietic stem cell transplant (alloHSCT) patients can contribute to cytomegalovirus (CMV) disease. CMV disease of the gut is associated with intestinal necrosis and ulceration and can lead to debilitating diarrhea. Diagnostic evaluation includes assessment for CMV viremia and intestinal biopsy to confirm diagnosis. Adequate research supporting intestinal biopsy in serum negative patients does not exist. In an effort to potentially minimize use of invasive endoscopic procedures to rule out CMV as a cause for diarrhea, we evaluated the diagnostic yield of intestinal biopsies in the work-up of allograft recipients presenting with diarrhea.

**Methods:** This retrospective study evaluated a total of 485 patients that underwent alloHSCT after 2006 and were admitted to the inpatient BMT service between January 1$^{st}$, 2008 and April 30$^{th}$, 2013. A subset of patients was identified that completed esophagogastroduodenoscopy (EGD) or colonoscopy for work-up of diarrhea. Comparisons were made between serum CMV PCR (108 bp primer directed at CMV Immediate Early antigen product) and gastrointestinal biopsy (with morphologic evaluation for cytopathic effect and immunohistochemistry for immediate early non-structural antigen). Pathologic evaluation confirmed gut-associated CMV disease.

**Results:** CMV viremia was evident the day of intestinal biopsy 25% (99 total biopsies) of the time. Nine biopsies (9%) in 7 different patients were positive for CMV and confirmed CMV gut disease. Of these, 6 patients had corresponding CMV viremia. One patient (cord blood recipient) was diagnosed with CMV gut disease by biopsy alone. Significant association (p=0.003) and agreement (p=0.006) between CMV viremia and CMV gut disease were observed in this cohort, although we do note discordances of interest. No apparent association between lymphocyte count and the presence of CMV intestinal disease was observed (p=0.23). In